

POLLEN AND PUBESCENCE CHARACTERISTICS
OF *OXALIS GRANDIS* SMALL

DARRELL MOORE, DALLAS MULLINS AND FOSTER LEVY

ABSTRACT

Pubescence in *Oxalis grandis* Small was analyzed as a constellation of characters describing the numbers, types, orientations, and lengths of hairs on different regions of the plants. Pubescence is a complex character whose components vary independently with no obvious geographical or ecological correlates. Hirsute plants occur sporadically throughout the range of the species, but extreme expression of the entire suite of pubescence characters is restricted to one population. A correlation analysis indicates that pubescence in *Oxalis grandis* is most likely under complex genetic control and is therefore valuable as a taxonomic character. An analysis of pollen size utilizing plants from two populations representing extremes in pubescence shows significant differences between flower morphs. For corresponding morphs, pollen from the population with pubescent plants is larger. These studies have demonstrated high levels of intraspecific polymorphism within *Oxalis grandis*.

Key Words: character analysis, heterostyly, *Oxalis*, pollen, pubescence

INTRODUCTION

The genus *Oxalis* comprises species with heterostylous and homostylous flowers. *Oxalis grandis* Small, a resident of the southern Appalachian Mountains and Ohio River Valley, is tristylous with marked size differences among the style lengths of its three flower morphs (Eiten, 1963). Each style morph has two whorls of functional stamens. Members of the species possess characteristics of subsection *Strictae*, section *Corniculatae*: a creeping rootstock, spreading pedicels and septate trichomes. *Oxalis grandis* is further distinguished by relatively large leaflets bordered by a purple margin (Wiegand, 1925). All individuals from a population inhabiting lower and middle slopes of bluffs along the Watauga River in Sullivan and Washington Counties of northeastern Tennessee are extremely hirsute on all parts of the plant and thus are distinguished easily from those of more typical populations (Levy and Moore, 1993). These morphological differences persist when plants are transplanted to a common greenhouse environment and in a growth chamber when new ramets are propagated from root cuttings (F. Levy and D. Moore, unpublished data).

The extreme intraspecific variation encompassed by the Wa-

tauga River population in comparison to more typical populations provided an opportunity for an in-depth examination of the pubescence phenotype. Our objective was to provide a full description of the morphological differences related to pubescence and to conduct a herbarium search for *Oxalis grandis* specimens with extreme amounts of pubescence. We then performed a character correlation analysis to assess whether pubescence is under simple or complex genetic control. Specific goals of the study were to determine whether (i) the extremely pubescent phenotype is unique to the Watauga River population, (ii) there are geographic or ecological correlates associated with pubescence, (iii) expression of components of pubescence vary independently. Consequently, we have dissected pubescence into several qualitative and quantitative components and examined the expression of each in individual plants.

In the most recent treatment of subsection *Corniculatae*, Eiten (1963) considered qualitative components of epidermal vestiture. Eiten's implicit assumption was that each component represented an independent character. If pubescence is a simple character with a simple genetic basis, or if it is environmentally determined, then it has minimal taxonomic value (Rollins, 1958). On the other hand, if pubescence represents the expression of several independent characters then it merits more attention in assessing degrees of differentiation. In this study, we use correlation analysis to assess the complexity of the pubescence phenotype and as a test of Eiten's assumption of independence.

A second objective of the study is an analysis of pollen sizes of the three flower morphs of *O. grandis* to determine whether pollen diameter differs among flower morphs and whether pollen size differs between populations. For this analysis we compared pollen from two populations, the Watauga River population and a population in a more typical habitat for the species (mesic forest at Iron Mountain Gap, Unicoi County, TN).

SECTION I—PUBESCENCE

METHODS

Pubescence was measured on ten plants from each of two study populations. Of these ten plants, four and three were fresh and the remainder recently collected and dried, from the Iron Moun-

tain Gap and Watauga River populations, respectively. Plants were carefully chosen to encompass the range of mature plant size, habitat and geographical location within each population. Only mature, flowering or fruiting specimens were included. The numbers of nonseptate hairs (no septate hairs occur on leaflets) were counted within 5×5 mm squares on the abaxial and adaxial leaflet surfaces. These grids were displaced a minimum of 2 mm from the midvein of one leaflet on each plant. For a subset of plants, hairs were counted on three different leaves, but preliminary analysis showed little variation and so, for the remainder, only one grid was counted per plant. Nonseptate and septate hairs also were counted on the circumference of one centimeter lengths of (i) the middle of a petiole, (ii) a section of the stem from the upper one-third of the plant (upper stem), and (iii) a section of the stem from the lower third of the plant (lower stem). Also measured were the maximal lengths of leaflet hairs (all nonseptate) and the septate and nonseptate hairs on stems. All counts and measurements were conducted at $40\times$ under a dissecting microscope.

The analysis identified a suite of eleven pubescence characters by which the two populations may be differentiated (Table 3). We measured the same set of characters in a survey of herbarium specimens to determine (i) whether the extremely pubescent phenotype of the Watauga River population was unique or if a continuum of variation existed within the species, and (ii) whether individual characters related to pubescence vary independently. For the detailed analysis, we chose only herbarium specimens possessing some hairs on their leaves, that is, specimens showing at least a slight tendency toward a hirsute phenotype. The screen for pubescent phenotypes included specimens from the following five herbaria: UNC (51 sheets), TENN (31 sheets), VPI (25 sheets), WVA (57 sheets), and the collection at EAST TENNESSEE STATE UNIVERSITY (10 sheets).

DATA ANALYSIS. For each of the five regions for which hairs were counted (abaxial and adaxial leaf blades, petioles, upper and lower stems), separate Kruskal-Wallis tests were conducted to compare the number of hairs between populations. This non-parametric test was chosen because of heteroscedasticity of variances among populations. Separate analyses were carried out for septate and nonseptate hairs. G tests were used to compare proportions of septate and nonseptate hairs. These tests were con-

ducted first within populations to determine whether proportions varied in different regions of plants and then corresponding regions of plants were compared among populations.

Associations between pairs of pubescence characters were estimated by Pearson Correlation Coefficients (CORR procedure, SAS Institute, 1982). These correlations were computed between all possible pairs of characters within: (i) the sample of 10 plants from the Iron Mountain Gap population, (ii) the sample of 10 plants from the Watauga River population and, (iii) the sample of 14 herbarium specimens exhibiting hairs on the leaves. Eight characters were included for the Iron Mountain Gap and Watauga River samples (there was no variation for any of the three hair-length characters within either population), and eleven characters in the herbarium sample (Table 3).

RESULTS

The Iron Mountain Gap population exhibited the complete absence of hairs on the abaxial and adaxial leaf surfaces, except for sparse nonseptate hairs along the midvein and leaflet margins. This contrasted strongly with the abundant nonseptate hairs on both leaf surfaces of all plants from the Watauga River population (Table 1). Although the midvein of leaflets from both populations had nonseptate hairs, those of the Watauga River population were longer (1.0–1.5 vs 0.7 mm) and were patent in contrast to the appressed hairs of the Iron Mountain Gap population. Both septate and nonseptate hairs occurred throughout the vegetative axis (stems and petioles) of plants from both populations, but the mean number of hairs was significantly greater in the Watauga River population for each hair type in each region of the plant (Table 1). Furthermore, the ranges of variation in the number of nonseptate hairs on petioles, as well as on upper and lower stems, did not overlap between the two populations (Table 1).

The ratio of septate : nonseptate hairs increased significantly on the lower stems within both populations, but did not differ between upper stems and petioles (Table 2). Comparisons between populations showed that the relative contribution of septate hairs on petioles was similar, but for upper and lower stems, it was significantly higher in the Iron Mountain Gap population (Table 2).

The unusual Watauga River population can be characterized

Table 1. Pubescence characteristics of two populations of *Oxalis grandis*. Entries represent the mean, standard deviation (SD) and range for numbers of septate and non-septate hairs in different regions of plants. The Kruskal-Wallis statistics and their associated probabilities (* <0.05, *** <0.001) are given for comparisons among populations.

	Stem				Petiole		Leaflet	
	Upper		Lower		Non-septate	Septate	Abaxial	Adaxial
	Non-septate	Septate	Non-septate	Septate				
Iron Mountain Gap								
Mean (SD)	53.3 (17.6)	25.4 (9.9)	22.2 (18.6)	69.1 (32.8)	45.7 (14.0)	15.0 (7.3)	0	0
Range	29–89	9–44	8–57	39–149	30–78	8–30	0	0
Watauga River								
Mean (SD)	375.0 (143.7)	67.8 (65.0)	250.2 (191.8)	203.3 (122.7)	209.1 (87.0)	39.9 (24.4)	79.9 (26.5)	131.3 (60.9)
Range	190–585	4–235	123–780	46–470	115–410	11–73	30–119	49–225
Kruskal-Wallis	14.3***	5.0*	14.3***	8.3***	14.3***	6.2*		

by a suite of traits that includes; abundant, long, patent, nonseptate hairs on the abaxial and adaxial leaf surfaces; and abundant, long, patent septate and nonseptate hairs on the stems. Thus, these plants have an easily observed dense covering of relatively long hairs throughout the plant; a hand lens is not necessary to distinguish this phenotype.

CHARACTER CORRELATIONS. In the Iron Mountain Gap population, four of the 28 possible correlations between pairs of characters were significant (Table 3). Several significant character correlations (seven of the possible 28) occurred in the Watauga River population (Table 3), and five of these were highly significant. The correlation matrix for the sample of herbarium specimens consisted of eleven characters (three hair-length characters were included in addition to the eight hair type characters). Six of the 55 possible correlations were significant (Table 3). All six significant correlations were positive and concerned characters related to numbers of nonseptate hairs. Six of the ten possible nonseptate/nonseptate correlations were significant. There were no significant correlations involving septate/nonseptate pairs of characters or any combination of hair length characters. In summary, there were significant correlations involving septate and nonseptate hairs within and between organs in the Watauga River sample, but in the herbarium sample, the only significant associations involved nonseptate hairs.

SECTION II—POLLEN

METHODS

Oxalis grandis is morphologically tristylous, that is, “the stigmas occur at a level different from the level of either of the two rings of anthers: either below both (short-styled), between them (midstyled), or above both (long-styled)” (Eiten, 1963). For most flowers, pollen was sampled from both anther whorls of each flower. Slides of pollen samples were prepared from fresh material in the field. A freshly dehisced anther was touched to a glass slide that was then stained with lactophenol aniline-blue dye. Because *Oxalis grandis* propagates clonally from rhizomes, sampled plants were separated by a minimum of 3 m. The samples from each population spanned the physical extent of each population and encompassed an area of approximately 5 km² and 20 km² in the

Table 2. Relative contribution of septate hairs (expressed as percent septate) to pubescence on different parts of *Oxalis grandis* in two populations. The value of the *G* statistic is given for each population comparison, asterisks refer to probabilities associated with each value of *G*. M = Iron Mountain Gap, W = Watauga River, ns = non-significant, ** <0.01, *** >0.001.

	Population		<i>G</i> (between populations)
	M	W	
Petiole	24.7%	16.0%	2.4 ns
Upper stem	32.3%	15.3%	10.4**
Lower stem	75.7%	44.8%	30.2***
Upper vs. lower stem (within populations)			
<i>G</i>	33.4***	95.8***	

Iron Mountain Gap and Watauga River populations, respectively. The diameters of ten pollen grains were measured from each anther using an ocular micrometer at 400 \times .

DATA ANALYSIS. For each style morph in each population, a separate one-way analysis of variance (ANOVA) was used to compare pollen diameters between the two anther whorls of a morph. None of these comparisons showed significant differences and therefore, anther samples from the two whorls of a flower morph were pooled for comparisons among style morphs within and between populations. Pollen diameters among style morphs within populations were compared by one-way ANOVA followed by Student-Newman-Keuls (SNK) comparisons among means (SAS Institute, 1982). To compare pollen diameters between populations, each morph was treated separately in a one-way ANOVA followed by SNK comparisons among means.

RESULTS

Differences in pollen diameter between anther whorls were not significant for any of the three flower morphs within either population, although sample sizes for some comparisons were low (Table 5). Nevertheless, there were significant differences in pollen diameter among flower morphs within each population. In both populations, pollen from the short-styled morph was significantly larger than pollen from the two other morphs (Table 6). For all three flower morphs, pollen from plants in the Watauga River population was larger than pollen from the corresponding morph

Table 3. Character correlations within (A) the Iron Mountain Gap population, (B) the Watauga River population, and (C) a sample of selected herbarium specimens of *Oxalis grandis*. For each significant correlation, the Pearson Correlation Coefficient and its associated probability is shown. Character correlations that were significant in two of the sample groups are denoted by a common symbol.

Correlated Characters	Coefficient	<i>P</i>	Shared Correlations
A. Iron Mountain Gap			
StUpNS:StUpS	0.68	0.03	*
StUpNS:StLoNS	0.73	0.02	#
StLoNS:StUpS	0.70	0.02	
StLoS:PetS	0.68	0.03	+
B. Watauga River			
AbLf:AdLf	0.81	0.004	o
AbLf:StLoNS	0.65	0.04	
StLoNS:PetNS	0.84	0.002	
StLoNS:StLoS	0.82	0.004	
StLoS:PetS	0.80	0.006	+
StLoNS:StLoS	0.82	0.004	
StUpNS:StUpS	0.63	0.05	*
C. Selected Herbarium Sample			
AbLf:AdLf	0.55	0.04	o
AdLf:PetNS	0.54	0.05	
AdLf:StUpNS	0.84	0.0001	
AdLf:StLoNS	0.82	0.0003	
PetNS:StUpNS	0.72	0.004	
StUpNS:StLoNS	0.58	0.03	#

Key to Acronyms: number of hairs, adaxial leaflet surface, AdLf; number of hairs, abaxial leaflet surface, AbLf; number of nonseptate hairs, petiole, PetNS; number of septate hairs, petiole, PetS; number of nonseptate hairs, upper stem, StUpNS; number of septate hairs, upper stem, StUpS; number of nonseptate hairs, lower stem, StLoNS; number of septate hairs, lower stem, StLoS; nonseptate hair length, abaxial leaflet surface, LfHr; nonseptate hair length, stem and petiole, StHrNS; septate hair length, stem and petiole, StHrS.

in the Iron Mountain population (Table 6) and these differences were highly significant for the mid- and long-style morphs (ANOVA results: short-styled, $F = 2.96$; $df = 1, 12$; $P = 0.11$; mid-styled, $F = 26.6$; $df = 1, 15$; $P = 0.0001$; long-styled, $F = 8.5$; $df = 1, 31$; $P = 0.006$).

DISCUSSION

PUBESCENCE. In a study of *Oxalis* section *Corniculatae*, Wiegand (1925) noted extreme intraspecific variation in pubes-

Table 4. Characteristics of herbarium specimens^a of *Oxalis grandis* that showed some leaf hairs. An “X” indicates expression of the character was within the range of variation observed within the Watauga River population. “Total W” indicates the number of Watauga River-like characters of the eight characters analyzed on each plant. “# Plants” refers to the number of test plants (max. = 14) that show the Watauga River-like phenotype for each character. C1 and C2 serve as controls since they were Watauga River site specimens collected prior to the current study. Acronyms as in Table 3.

Plant	Character								Total W
	AbLf	AdLf	PetNS	StUpNS	StLoNS	LfHr	StHrNS	StHrS	
1.	X								1
2.				X					1
3.					X				1
4.								X	1
5.	X		X						2
6.	X		X						2
7.	X					X			2
8.	X		X		X			X	4
9.	X	X	X	X	X				5
10.	X	X		X	X	X			5
11.	X	X	X	X				X	5
12.	X	X		X	X	X		X	6
13.	X	X	X	X	X	X			6
14.	X	X	X	X	X	X			6
C1.	X	X	X	X	X	X	X	X	8
C2.	X	X	X	X	X	X	X	X	8
# Plants	11	6	7	7	7	5	0	4	

^a Key to herbarium specimens: 1. VIRGINIA, Scott Co., summer 1958, *E. Elliott s.n.* (EAST TENNESSEE STATE UNIVERSITY); 2. VIRGINIA, Wythe Co., open woods, 28 June 1910, *FSH s.n.* (VPI); 3. KENTUCKY, Rockcastle Co., road bank along Rockcastle River, 17 June 1961, *H. E. Ahles 54501 with H. Smith* (UNC); 4. KENTUCKY, Pulaski Co., mixed hemlock-hardwoods, Daniel Boone National Forest, 23 July 1970, *E. M. Browne and E. T. Browne Jr. 70K15.19* (UNC); 5. TENNESSEE, Unicoi Co., Unaka Island, 8 June 1974, *C. L. Shepard 168* (EAST TENNESSEE STATE UNIVERSITY); 6. WEST VIRGINIA, Hancock Co., Newmans Bridge, 21 June 1963, *J. Bonar s.n.* (WVA); 7. KENTUCKY, Menifee Co., Red River Gorge, rich moist woods, 28 May 1969, *P. D. Higgins 1401* (UNC); 8. WEST VIRGINIA Raleigh Co., 25 May 1940, *J. P. Tosh s.n.* (WVA); 9. TENNESSEE, Pickett Co., north-facing bluff of Wolf River, 3 May 1984, *G. L. Walker, E. E. C. Clebsch, Z. E. Murrell 017* (TENN); 10. KENTUCKY, Edmonson Co., mesophytic woods beside Bylew Ck., 7 June 1968, *K. A. Nicely and H. W. Elmore 1718* (UNC); 11. INDIANA, Floyd Co., shale knobs, 18 May 1963, *A. C. Koelling 1065* (TENN); 12. TENNESSEE, Cocke Co., roadside near Douglas Lake, 3 May 1966, *B. Allen s.n.* (TENN); 13. ALABAMA, Colbert Co., rich north-facing slope over sandstone, 24 May 1974, *R. D. Whetstone and T.*

Table 5. ANOVA comparisons of pollen diameters (microns) between anther whorls within style morphs of *Oxalis grandis*. *n* = number of anthers sampled.

Morph	Anther Whorl	<i>n</i>	Mean	<i>F</i>	<i>P</i>
Iron Mountain Gap					
Short	mid	2	35.45	0.07	0.80
	long	3	34.91		
Mid	short	6	29.85	0.22	0.65
	long	6	30.53		
Long	short	12	32.90	1.70	0.21
	mid	11	31.42		
Watauga River					
Short	mid	1	41.60	NA	—
	long	2	37.12		
Mid	short	2	39.81	5.20	0.11
	long	3	35.54		
Long	short	4	34.14	2.01	0.19
	mid	6	35.41		

cence that he assumed arose from environmental and soil factors. The exception, *O. europea* Jord. (= *O. stricta* L.), had stem and pedicel pubescence that Wiegand attributed to the environment, but on the basis of geographical correlates, he postulated that hairiness on the abaxial leaf surface represented racial differentiation. In the most recent and thorough treatment of the section, Eiten (1963) contradicted Wiegand's views and relied upon variability in pubescence as a key diagnostic character in dividing section *Corniculatae* DC into subsections *Corniculatae* and *Strictae* Eiten. The diagnostic characteristics of the latter subsection were (i) the presence of septate hairs on stems, petioles and pedicels, and (ii) stems that arose singly from thin underground rhizomes (Eiten, 1963).

In contrast to our observations, prior studies have concluded that leaf hairs in *Oxalis grandis* are rare. Wiegand's (1925) description of *O. grandis* included presence of sparing pubescence

←

Atkinson 3198 (UNC); 14. TENNESSEE, Sinking Creek, moist woods, 11 June 1955, *J. Pearman s.n.* (EAST TENNESSEE STATE UNIVERSITY); C1. TENNESSEE, **Washington Co.**, shale; open woods, shale barren-like, 16 May 1990, *K. Renzaglia s.n.* (VPI); C2. TENNESSEE, **Washington Co.**, Watauga Flats, 5 May 1978, *T. Bruce s.n.* (EAST TENNESSEE STATE UNIVERSITY).

Table 6. ANOVA comparisons of pollen diameters (microns) among style morphs of *Oxalis grandis* with SNK comparisons of mean diameters. Means followed by the same superscript were not significantly different ($P < 0.05$).

Iron Mountain Gap				
Source	df	MS	<i>F</i>	<i>P</i>
Among morphs	2	74.69	9.83	0.0003
Error	43	7.60		
Morph	<i>n</i>	Mean		
Short	11	35.25 ^a		
Long	12	32.19 ^b		
Mid	23	30.19 ^b		
Watauga River				
Source	df	MS	<i>F</i>	<i>P</i>
Among morphs	2	19.85	4.44	0.03
Error	15	4.47		
Morph	<i>n</i>	Mean		
Short	3	38.61 ^a		
Mid	5	37.25 ^{a,b}		
Long	10	34.91 ^b		

on the stems, villous petioles and peduncles, and glabrous leaves except for a few hairs on the underside; he did not distinguish septate from nonseptate hairs. Similarly, in an exhaustive study of section *Corniculatae*, Lourteig (1979) concluded that pubescence is rarely found on the leaf blades in *O. grandis*. Both studies noted a specimen (Cincinnati, C. G. Lloyd, 1882) that was unusually hairy on the stem with strigose abaxial leaf surfaces. Our results indicate that leaf blade pubescence occurs sporadically throughout the range of the species because nonseptate hairs were seen on abaxial leaf surfaces in 16 of 174 herbarium specimens examined.

Our results suggest that in *Oxalis grandis*, four different types of pubescence characters contribute to the extremely hirsute phenotype—an increase in the number of (1) septate and (2) nonseptate hairs, (3) elongation of both types of hairs throughout the vegetative axis and leaves, and (4) reorientation of hairs from appressed to patent. All except the last of these distinguishing characteristics are quantitative, and in only one of these (length of nonseptate hairs on the vegetative axis) is the extreme phe-

notype (long hairs) restricted to the Watauga River population. For the remaining quantitative characters, intermediate and extreme phenotypes occur either as isolated characters, or in various combinations.

Long, nonseptate hairs on stems occurred only in the Watauga River population (Table 4, StHrNS, specimens C1, C2). Considering the sample of herbarium specimens, there were no correlations involving the other two hair-length characters (LfHr; StHrS). However, in individual specimens, these two characters occurred singly (Table 4, specimen #4), in combination with each other (Table 4, specimen #12), or in various, apparently random combinations with other characters. From the apparent independence of these characters, we conclude that the hair length component of the Watauga River phenotype is a complex character.

In the screened herbarium sample, none of the character correlations involving septate hairs was significant. Nevertheless, the hirsute (Watauga River) phenotype was observed in eight specimens for number of septate hairs on petioles, six specimens for number of septate hairs on upper stems, and six specimens for number of septate hairs on lower stems (data not shown). In two specimens (Table 4, specimens #10, #13), septate hairs were absent on the upper stem, indicative of an extreme Iron Mountain Gap phenotype, but the number of nonseptate hairs was clearly typical of the Watauga River phenotype. In other herbarium specimens, nonseptate hairs were sparse (Iron Mountain Gap phenotype) but septate hairs were abundant (Watauga River phenotype) in corresponding regions of the plants. Two lines of evidence suggest that an increased number of septate hairs is independent of other pubescence characters. First, there were no significant character correlations involving septate hairs in the herbarium sample. Second, hirsuteness for septate hairs occurred in combination with extreme variation (encompassing both phenotypes) in the numbers of nonseptate hairs.

In the herbarium sample, there were no obvious ecological or geographical correlates associated with the occurrence of pubescent plants. Because herbarium sheets rarely contain more than a few individual plants, it is not possible to know if pubescent plants represent unusual plants or if, like the Watauga River population, the entire population is fixed for some increased degree of hairiness. Nevertheless, the observation that plants representing both extremes of hairiness retained their characteristic phe-

notypes after propagation in a common environment indicates a genetic basis (Levy and Moore, 1993).

Although character correlations exist within the Iron Mountain Gap sample (Table 4), these plants showed little tendency toward the hirsute phenotype. Therefore, the meaning of these particular correlations is unclear. The Watauga River population provided the standard by which we defined the extreme hirsute phenotype with multiple positive associations among pubescence characters. However, the sample of selected herbarium specimens may provide a key to understanding the complexity of pubescence in *Oxalis grandis*. These specimens were selected because each showed a tendency towards hairiness. Within this sample, all characters involving nonseptate hairs were positively correlated, suggesting simple genetic control may underlie the increased number of nonseptate hairs. On the other hand, numbers of septate hairs appeared independent of all other characters, and therefore is probably under independent genetic control. Furthermore, neither of the two hair-number characters were correlated with increased length of either type of hair.

If one considers only qualitative differences between phenotypic extremes (e.g., patent versus appressed hairs; short versus long nonseptate hairs), then it could be concluded that pubescence is a simple character. For example, in *Dithyrea* (Rollins, 1958) and *Linum* (Rogers, 1968) hairiness is controlled by a single genetic locus. However, in *Oxalis grandis* we have provided several lines of evidence involving qualitative as well as quantitative pubescence attributes suggesting a more complex character. In the herbarium sample we found (i) only one significant character correlation in common with the Watauga River population, (ii) no significant correlations between septate and nonseptate hairs and, (iii) no individuals with long nonseptate hairs on stems or petioles (unlike their ubiquitous occurrence in Watauga River). The apparent complexity of the pubescence character in *Oxalis grandis* endows it with significant value as an indicator of taxonomic relationship but, the contrast between *Dithyrea* and *Oxalis* highlight the necessity of a detailed character analysis for each taxon under study.

POLLEN SIZE. Citing data from his own crosses, as well as from Muller and Hildebrand, Darwin (1877) convincingly showed that illegitimate pollination of several heterostylous *Oxalis* species resulted in dramatically reduced seed production compared

to legitimate pollinations. In *O. regnellii*, *O. speciosa*, and *O. valdiviana*, pollen diameter was correlated with the stamen whorl from which it was produced. The largest grains were produced in long stamens, whether these stamens occurred on short- or mid-styled flower morphs; the smallest pollen was found in short anthers. Pollen in *Oxalis grandis* also is heteromorphic. In both populations of *Oxalis grandis* examined, short-styled morphs produced the largest pollen (Table 5). However, pollen diameter in this tristylous species is largely a function of the style morph on which it was produced; pollen from different anther whorls within a style morph are similar in size.

Several heterostylous species in *Oxalis* subsection *Corniculatae* have lost the self-incompatibility response and others show evidence of loss of intermorph morphological differences (Ornduff, 1972). The three closely related species that constitute *Oxalis* subsection *Strictae* (*O. grandis*, *O. stricta*, *O. suksdorfii*) are all moderately to strongly self-compatible (Ornduff, 1964, 1972). Moreover, pollen size in this section of the genus is not clearly related to the length of the anther whorl. For example, long anthers on short- and mid-styled morphs in *O. suksdorfii* produced the largest pollen, but there was no difference in pollen size between anther whorls in long-styled morphs. The size of pollen grains from corresponding anthers on different style morphs differed within long and mid anther whorls (Ornduff, 1964). Mid-styled flowers showed strong pollen size differences between anther whorls that was accompanied by physiological differences in the incompatibility reaction (Ornduff, 1964).

In some species of *Oxalis*, differences in pollen size have been noted between populations as well as among anther whorls. For example, pollen diameter differed among anther whorls from two style morphs within each of three populations of *O. dillenii* ssp. *filipes*, but the morph that lacked pollen size differentiation among anther whorl types was not the same in each population (Ornduff, 1972). *Oxalis alpina* (section *Ionoxalis*) consists of di- and tristylous populations. In the former, style morphs clearly differed in the size of pollen produced, but there were no size differences among anther whorls within style morphs (Weller, 1976). Pollen appeared trimorphic in tristylous populations, but the magnitude of differences between morphs was not as pronounced as in distylous populations (Weller, 1976). A detailed analysis of the trimorphic pollen of *Oxalis pes-caprae* showed variation among

populations when pollen from equivalent anthers was compared (Ornduff, 1987). In the present study, pollen size was remarkably different between the Watauga River and Iron Mountain Gap populations within each flower morph. Pollen from the Watauga River population always is larger and, for the mid- and long-styled morphs, these differences are highly significant.

CONCLUSIONS

Two lines of evidence suggest that, in *Oxalis grandis*, pubescence is genetically controlled. In prior studies, distinct pubescence phenotypes retained their respective suites of characteristics in a common environment. The current study uncovered the sporadic occurrence of plants with varying degrees of a pubescent phenotype, but these plants did not share obvious geographical or ecological factors. Furthermore, the correlation analysis has shown that components of pubescence vary independently in nature. While a determination of the genetic basis of pubescence is premature without a formal genetic analysis, the current findings suggest a genetic architecture more complex than a single locus. We postulate that the constellation of characters leading to an extremely pubescent phenotype may arise from either the combined actions of a minimum of three loci with major effects or it may result from several polygenic characters. The complexity of the phenotype tends to lend credence to Eiten's emphasis on pubescence as a diagnostic character in *Oxalis* section *Corniculatae*.

Copious, long patent hairs and relatively large pollen grains indicate the Watauga River population is an extreme variant of *Oxalis grandis*. This population also differs in other attributes from more typical representatives of the species. For example, there were distinct differences from the Iron Mountain phenotype with respect to the pattern of its circadian rhythm of leaflet sleep movements as well as its responses to light level fluctuations (Levy and Moore, 1993) which suggest some fundamental physiological diversity. Finally, the Watauga River population occupies an unusual habitat—steep slopes underlain by easily eroded shale of the Sevier formation. The resultant substrate and habitat is reminiscent of the shale barrens of Virginia and West Virginia. These sites are known to support regionally rare species and some areas recently have been afforded protection. Future studies may show

that the extremely pubescent phenotype is worthy of varietal status but, for the present, we have chosen to adopt a conservative approach and retain *Oxalis grandis* as a monotypic species but to recognize its morphological and physiological diversity.

ACKNOWLEDGMENTS

We gratefully acknowledge support from the East Tennessee State University Research Development Committee. We gratefully acknowledge the courtesy of the curators of TENN, UNC, VPI, and WVA for allowing examination of specimens from their collections.

LITERATURE CITED

- DARWIN, C. 1877. The Different Forms of Flowers on Plants of the Same Species. John Murray, London.
- EITEN, G. 1963. Taxonomy and regional variation of *Oxalis* section *Corniculatae*. I. Introduction, keys and synopsis of the species. Amer. Midl. Naturl. 69: 257–309.
- LEVY, F. AND D. MOORE. 1993. Population variation of leaflet sleep movements in *Oxalis grandis* (Oxalidaceae). Amer. J. Bot. 80: 1482–1493.
- LOURTEIG, A. 1979. Oxalidaceae extra-austroamericanae. Phytologia 42: 57–198.
- ORNDUFF, R. 1964. The breeding system of *Oxalis suksdorfii*. Amer. J. Bot. 51: 307–314.
- . 1972. The breakdown of trimorphic incompatibility in *Oxalis* section *Corniculatae*. Evol. 26: 53–65.
- . 1987. Reproductive systems and chromosomal races of *Oxalis pes-caprae* L. and their bearing on a noxious weed. Ann. Mo. Bot. Gard. 74: 79–84.
- ROGERS, C. M. 1968. The *Linum bahamense* complex. Rhodora 70: 439–441.
- ROLLINS, R. C. 1958. The genetic evaluation of a taxonomic character in *Dithyrea* (Cruciferae). Rhodora 60: 145–152.
- SAS INSTITUTE. 1982. SAS User's Guide: Statistics. Research Triangle Park, NC.
- WELLER, S. G. 1976. Breeding system polymorphism in a heterostylous species. Evol. 30: 442–454.
- WIEGAND, K. M. 1925. *Oxalis corniculata* and its relatives in North America. Rhodora 27: 113–124, 133–139.

DEPARTMENT OF BIOLOGICAL SCIENCES
EAST TENNESSEE STATE UNIVERSITY
JOHNSON CITY, TENNESSEE 37614